[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

A Histopathological Study of Experimental Purulent Meningo-encephalitis in the Mouse *

David A. Carlson
B.A. Bowdoin College
1954

A Thesis Presented to the Faculty of the Yale School of Medicine in Candidacy for the Degree of Doctor of Medicine

Department of Pathology
School of Medicine
Yale University
1958

* This work was supported in part by grants from the James Hudson Brown Memorial Fund and the National Science Foundation.

ACKNOWLEDGMENTS

The author wishes to express his gratitude to Dr. Elias E. Manuelidis, Assistant Professor of Pathology in the Yale University School of Medicine for his guidance and active assistance in the preparation of this work.

The author also wishes to thank Miss Betty Mallaly for her generous and invaluable technical assistance.

ACKNOWLEDGEMENTS

The author wishes to express his gratitude to
Dr. Elias K. Manousidis, Assistant Professor of
Pathology in the Yale University School of Medicine,
for his guidance and active assistance in the pre-
paration of this work.

The author also wishes to thank Miss Judith Mullaly
for her generous and invaluable secretarial assistance.

INTRODUCTION

During the course of an experimental study of West Nile virus infection in mice involving the intracerebral injection of the virus a bacterial contamination occurred. Smears of brain surfaces showed gram-positive cocci; and cultures of both swabs and brain homogenate grew out alpha-hemolytic streptococcus. Mice infected in this way showed a surprisingly virulent course, most of the animals dying between twelve and twenty four hours after injection and many others before twelve hours. Pathological examination revealed a purulent meningoencephalitis.

The severity of the infection, the rapid demise of the animals, the manifold pathologic changes, and a paucity of data on the morphological aspects of experimental bacterial infections of the central nervous system prompted an experimental study using this contaminant.

INTRODUCTION

MATERIAL AND METHODS

Two hundred female white mice three months of age were injected intracerebrally with .03 cc. of a 10% suspension of mouse brain contaminated with alpha-hemolytic streptococci. Another group of fifty similar animals received .03 cc. of an eight-day blood broth culture of alpha-hemolytic streptococcus. There was no difference in the courses or findings of the two groups, which henceforth will be considered together.

Each animal was maintained until its condition was terminal, as usually evidenced by complete failure of locomotion in response to stimulation and inability to right itself, in an attempt to demonstrate the most advanced lesions obtainable. In a few cases, when an animal was found dead within thirty minutes of a previous observation, the material was included. Sacrifice was performed with ether.

The brain and as much of the spinal cord as could be dissected in good condition were immediately fixed in formalin or bromformalin. Tissue embedded in paraffin was stained with hematoxylin and eosin and with Nissl technique. Other tissue, fixed in bromformalin, was cut with the freezing microtome and stained for microglia, and for astrocytes with Scharenburg's modification of silver carbonate,

MATERIAL AND METHODS

Two hundred lambs while nine weeks months of age were injected intraperitoneally with .1 cc. of a 10% suspension of mouse brain emulsified with alpha-hemolytic streptococci. Another group of fifty similar animals received .01 cc. of the eight-day blood broth culture of alpha-hemolytic streptococcus. There was no difference in the course in either of the two groups, which henceforth will be considered together.

Each animal was temperatured until the condition was terminal, as usually evidenced by complete inability of locomotion in response to stimulation and inability to right itself, in an attempt to demonstrate the most advanced lesion obtainable. In a few cases, when an animal was found dead within thirty minutes of previous observation, the autopsy was conducted. Sacrifice was performed with ether.

The brain and as much of the spinal cord as could be dissected in each case was fixed in formalin or bromalin. Tissue sections in paraffin was obtained by the Gomori-Lillie method and with Nissl technique. Frozen sections were made in bromalin, and cut with the Spencer-Riker and stained for fibers, and for myelin with Sudan III, Scharpburg's modification of Weigert's

while alternate sections of the block were stained with hematoxylin and eosin and Nissl stain.

The material presented is a series of composite pictures of findings at stated intervals following injection. Where exceptions to the general observations at any given interval occurred, they are noted and outlined.

RESULTS

12 - hour stage:

A purulent meningitis is seen. This is most marked in the midline of the forebrain and over the dorsal surface of the medulla, but is only patchy over the cerebellum. The cells are almost entirely polymorphonuclear. There are focal hemorrhages surrounded by intense leukocytic infiltrates in the cerebral meninges. Leukocytic infiltrates involve many areas of the cerebral cortex as meningitic extenstions; and in many such instances the limiting glial membrane is found in silver stain to be apparently intact.

There is ventriculitis with necrosis of the ependyma, of portions of the choroid plexus, and of much of the periventricular region. (Fig. 1). This condition extends from the lateral ventricles through the fourth ventricle. The necrosis is symmetrical, and its typical picture is one of 1) a pale strip of complete necrosis, 2) a zone of hemorrhage in some cases accompanied by large numbers of polymorphonuclear cells, 3) a zone of markedly swollen astrocytes (Fig. 2), and 4) a zone of microglial proliferation. The last two zones overlap to varying degrees; but their order is constant. In some regions no polymorphonuclear leukocytes are to be seen in the

12 - Four stage:

second zone. Perivascular polymorphonuclear infiltrates are seen along some vessels in the last three of these zones and sometimes beyond them, but are inconstant. In one area near the necrosis surrounding the third ventricle a focus of demyelinization is observed; and in one area the thin strip of necrosis bordering the ventricles is replaced by a broad zone of loculation (Fig. 3).

Bilateral symmetrical areas of necrosis and hemorrhage are seen in the rhinencephalon. This is accompanied by mild to moderate perivascular infiltrates and by scattered polymorphonuclear cells in the parenchyma. A similar though unilateral lesion is seen in the granular layer of the cerebellum; and an area of necrosis and hemorrhage with minimal leukocytic response is seen in the thalmus.

The base of each hemisphere contains a few scattered abcesses.

In the basal ganglia there is a circumscribed area of pallor and sponginess in which the cellular elements are relatively well preserved.

In some areas beneath the meningitis where there is no extension of the process into the cortex, the astrocytes of the superficial cortical layer are pale and slightly swollen in hematoxylin-eosin preparations without accompanying microglial changes.

However, in other areas the astrocytes in addition to pallor and swelling reveal peripheral chromatic condensation and are accompanied by twisted microglia cells. Where cortical leukocytic infiltrates are found the only change consists in a few pale, swollen astrocytes. In the molecular layer of the cerebellum pallor and swelling of the astrocytes are seen consistently and are often independent of the overlying meningitis and of extensions of meningeal inflammation to the cerebellum.

Pallor and swelling of the astrocytes are very often seen around the previously described hemorrhagic and necrotic foci, in some instances without microglial reaction. A few shadow forms of astrocytes are found near the periventricular areas of necrosis.

Hypertrophic, elongated, often twisted or rectangular forms of microglia are observed beneath the meningitis, particularly in the forebrain.

18 - hour stage:

In one animal whose condition appeared terminal at this interval there is a diffuse meningeal infiltration which is heavier than that observed at twelve hours. The cells are overwhelmingly polymorphonuclear.

The brain contains large areas of complete necrosis. Large numbers of bacteria can sometimes

be seen in such areas, often with only a very weak polymorphonuclear leukocytic response.

The injection tract is visualized and shows 1) a pale strip of complete necrosis, 2) a hemorrhagic zone with polymorphonuclear leukocytes and twisted, proliferated microglia, and 3) a zone of disintegrated astrocytes with decreased chromatin and process fragmentation as seen with silver stain. Some astrocytic nuclei in the last zone are swollen in hematoxylin-eosin preparations, while others are normal in size or slightly shrunken. The cellularity of zone 2, caused by the presence of polymorphonuclear leukocytes, obscures a full evaluation of the status of its astrocytes; but swollen cell bodies are discernible. Astrocytes beneath the meninges and in scattered areas throughout the parenchyma appear pale and, in some cases, slightly swollen. These changes are observed both with and without obvious relation to inflammatory and necrotic foci.

There is no circumscribed proliferation of microglia; but there is an equivocal increase of microglia throughout the tissue. One vessel appears to be surrounded by twisted and linear microglia forms.

No ventriculitis is seen.

20 - hour stage:

The meningitis is similar to that at eighteen hours, although in one case it is patchy in distribution. The spinal cord reveals meningitis more pronounced in the dorsal than in the ventral half; and in one case there is massive extension of the inflammation into the cord through an apparently intact limiting glial membrane, similar to findings described at twelve hours.

In one case bilateral symmetrical hemorrhages are again seen, this time in the mid cerebrum; and an abscess surrounds a vessel near one of the hemorrhages. The same brain contains a bilaterally symmetrical leukocytic infiltrate which seems to follow fiber tracts. Perivascular polymorphonuclear infiltrates are seen 1) in relation to the meningitis, 2) deep in the parenchyma, especially in the cerebellum, which is massively involved, and 3) near the injection tract. Scattered, well formed, fresh abscesses are seen in the cerebrum and, in greater numbers, in the cerebellum.

Ventriculitis in each case is without clear cut inflammatory extension into the subependymal tissue; but below the ependyma there is mild swelling of the astrocytes. In one case this swelling is accompanied by vacuolization and by absence of the astrocytic processes as seen in silver stain. In

20 - Four stage:

this preparation the astrocytes do not appear to be well preserved (Fig. 4).

Astrocytic changes beneath the meninges vary from place to place. Pallor and swelling (Fig. 5) are often seen in the absence of any inflammatory lesion, while changes in the presence of cortical leukocytic infiltrates vary from pallor and mild swelling to fragmentation of all processes and cellular destruction. The degree of astrocytic change in these cortical regions seems fairly consistent for each brain: e. g., if only mild swelling and pallor of astrocytes is seen in relation to one cortical infiltrate the same picture will probably be found around any other such infiltrate. This is also true of the astrocytic changes seen below areas of ventriculitis. However, the changes are less marked in the latter location.

Astrocytes surrounding the larger abscesses show marked alterations, ranging from swelling and pallor to a "moth eaten" appearance associated with process fragmentation, in which cases the cell bodies are of roughly normal size.

In the pronounced involvement of the spinal cord mentioned above, some astrocytes reveal a marked reduction of their processes immediately subjacent to the leukocytic infiltrations, while other astro-

this preparation the astrocytes do not appear to be
well preserved (Fig. 4).

Astrocytic changes beneath the meninges vary
from place to place. Sections are modified (Fig. 5)
are often seen in the absence of any inflammatory
lesion. While changes in the composition of cerebral
leukocytic infiltrates vary from diffuse mild
swelling to fragmentation of all components
cellular degeneration. The degree of astrocytic
change in these cortical regions seems directly
associated with each brain: e.g., if only mild
and patchy of astrocytes is seen in cortical
cortical infiltrates the same is true of the
be found are no any other such infiltrates.
also true of the astrocytic changes seen
areas of ventricularis. However, the changes
less marked in the lytic reaction.

Astrocytes proximate the lesion in close
sbon marked inflammation, varying from swelling
pallor to a "moth eaten" appearance. Many
process fragmentation. Some
are of normally normal (Fig.

In the more chronic lesions of
reduction of their processes (Fig. 7) and
to the leukocytic infiltration. While other

cytes are large and round to slightly oval without demonstrable processes. In silver preparations some of the nuclei of such cells reveal a reticular pattern, while other muclei show a peripheral condensation of silver stained material. Alternate sections of the spinal cord in hematoxylin-eosin demonstrate moderate astrocytic swelling.

In the case with diffuse cerebellar involvement, the astrocytes of the cerebellum are poorly stained and have only swollen, clumped cell bodies or, at most, a few swollen, irregular processes.

Where perivascular infiltrates are seen, the adjacent astrocytes are poorly demonstrable in silver.

Slight to moderate microlial proliferation is found in relation to hemorrhage and, in one animal, in the cerebellar cortex beneath the meningitis.

22 - hour stage:

There is a diffuse meningitis most marked over the surface of the cerebellum. The base of the pons contains bilaterally symmetrical extensions of the meningitic process: elsewhere there are the small cortical infiltrations previously seen. The exudates are composed of polymorphonuclear leukocytes.

Throughout the tissue are abscesses of varying

cytes are large and round to slightly oval bipolar
demonstrable processes. In silver preparations some
of the nuclei of such cells appear to be enlarged
pattern, while other nuclei show a pronounced con-
densation of silver stained material. Electron
sections of the animal used in Bielschowsky-Gros
demonstrate moderate endoplasmic reticulum.

In the case with Nissl preparations,
the astrocytes of the cerebellum are mostly resting
and have only scanty, stained cell bodies or, at
most, a few small, irregular processes.

Nissl preparations indicate the same. The
adjacent astrocytes are poorly demonstrable in
silver.

Slight to moderate microglial reaction is
found in relation to degenerating cells.

in the cerebellar cortex being the most intense.

S2 - hour stage:

There is a diffuse neuronal loss involving
the surface of the cerebellum. In areas of the
contains pigmented cytoplasm. Occasional
meningitic reaction: numerous lymphocytes in the
cortical infiltrations consisting of
are confined to perivascular locations.

There is the same type of reaction

size and random leukocytes in no clear relation to blood vessels. No truly perivascular infiltrates are found. Small hemorrhages are seen in the cerebellar white matter.

There is no ventriculitis.

Pallor with minimal to absent swelling of the astrocytes is seen in scattered fashion throughout the parenchyma, often without relation to any other change. Similar astrocytes are found in immediate relation to some of the less dense leukocytic infiltrates, while in the vicinity of discrete, focal polymorphonuclear accumulations in the pons and in the cerebral cortex, pallor is accompanied by definite swelling.

Proliferation of microglia is seen at this time. Isolated, elongated forms are seen in a distribution generally corresponding to that of the pale, scattered astrocytes described above. There are areas of isolated microglial proliferation, which are most common in the cerebellar molecular layer (Fig. 7). Surrounding focal leukocytic infiltrates in the pons there is proliferation of microglia as well as pale and swollen astrocytes. In sections corresponding to one half of the pons progressive changes of pre-existing microglia cells are observed, while in the contralateral half there is a definite microglial

size and random leukocytes in the deep portion of
blood vessels. No true basophilic material was
found. Small hemorrhages are seen in the deeper tis-
white matter.

There is no reaction

Pallor with thinning of adjacent portion of
astrocytes is seen in stained frozen sections of
the paraffin, often allowing retaining of the cyto-
charge. Similar reactivities are found in comparable
relation to removal that tissue [illegible] and
brates, while in the vicinity of [illegible].
rolymorphonuclear accumulations in the [illegible]
the cerebral cortex, pallor is more marked at pe-
ripheral.

Proliferation of [illegible] is seen in [illegible]
isolated, elongated forms are seen in [illegible]
generally corresponding to that of the [illegible].
astrocytes described [illegible]. Those are seen
isolated microglial proliferation,
common in the cerebellar [illegible] (Pl. 3).
Surrounding local [illegible] [illegible] is the
there is proliferation of [illegible]
and swollen astrocytes. In a distant [illegible]
one half of the [illegible]
existing disparity [illegible]
compressed [illegible]

proliferation as seen in the increased number of these cells.

26 - hour stage:

The meningitis is diffuse and again is most marked over the cerebellum. There are small cortical extensions in the form of leukocytic infiltrates; and these are most common in the cerebrum. The meningeal exudate is composed of polymorphonuclear leukocytes.

There are scattered parenchymal hemorrhages, which are now surrounded by clear cut layers of polymorphonuclear leukocytes. Similarly, there are scattered leukocytic foci without relation to hemorrhage or the meninges: such areas are most common in the molecular layer of the cerebellum.

There is no ventriculitis.

Astrocytes show diffuse involvement throughout much of the tissue, as reflected in pallor and swelling of cell bodies, chromatin condensation, and a reduction in cell processes, which number one or two at most and are thick and fragmented, or are completely lacking.

Binucleate astrocytes and apparent amitotic division are seen in one animal near one of the hemorrhages and in several foci 1-2 mm. beneath the meningeal infiltrate.

There is a diffuse increase in microglia without relation to hemorrhage, infiltration, or vessels.

28 - hour stage:

There is a heavy, diffuse meningitis which is most intense over the cerebellum and the medulla. There is still a definite predominance of polymorphonuclear leukocytes. Cortical extensions are seen as previously, with the exception that in a few areas the superficial limiting membrane no longer appears intact.

Scattered, well formed abscesses (Fig. 8) are seen throughout the brain tissue. Less well formed abscesses are seen in the cerebellar white matter, while the granular layer contains a necrotic focus and the molecular layer scattered polymorphonuclear leukocytes.

There is ventriculitis with bilateral, symmetrical necrosis of some of the periventricular structures, the necrotic zone being outlined by leukocytic infiltrates (Figs. 9, 10, 11).

Astrocytes stained in alternate fashion with hematoxylin-eosin and with silver, immediately subjacent to the meningitit are 1) apparently normal; 2) pale without swelling and with well-stained, intact processes; 3) pale and swollen with intact

processes; 4) pale and swollen with a decreased number of short, clumped processes; and 5) without intact processes (Fig. 12). Still another type of cell, with clumped cell body and fragmented processes, is seen in close relation to abscesses and on the border of the periventricular necrosis. This type of astrocytic alteration does not appear different in hematoxylin-eosin preparations from the astrocytic changes described above: i.e., the cells appear swollen and pale. Often these astrocytic changes in hematoxylin-eosin are not accompanied by any marked alterations in corresponding silver stained sections.

In general, the demonstration of astrocytic processes improves directly with distance from infiltrate, hemorrhage, or necrosis (Fig. 13,14).

The molecular layer of the cerebellum contains some pale, slightly swollen astrocytes.

Astrocytic changes may be seen with or without microglial reaction. Surrounding some areas of astrocytic changes beneath the meningitis there are reactive microglial forms. In other such areas twisted microglial cells are seen, intermingled with astrocytic changes. In still other areas, particularly beneath those portions of the limiting membrane which appear destroyed, microglia are apparently the

only intact cells. Circumscribed microglial proliferation is seen about one abscess (Fig. 15). There is a definite increase of microglia in the molecular layer of the cerebellum. Surrounding the zones of periventricular necrosis and beyond it, there are reactive forms of microglia.

Demyelinization and axon fragmentation are seen in the vicinity of one abscess.

32 - hour stage:

Meningitis is heavy, but patchy in some areas, and is marked more by round cells than by polymorphonuclear leukocytes. Cortical infiltrates are present.

In the parenchyma scattered hemorrhages and well-formed abscesses are again found. There is no ventriculitis.

Astrocytic changes are similar to those at twenty-eight hours. No proliferative forms are seen.

There is a stronger, diffuse increase of microglia in the molecular layer of the cerebellum, as well as more striking foci of proliferation under the meningitis.

DISCUSSION

A severe purulent meningitis can be observed in mice which were dead or moribund and therfore sacrificed twelve or eighteen hours after inoculation with hemolytic streptococci. Twelve hours after inoculation the meningitis is especially pronounced over the dorsal surface of the medulla and in the midline of the forebrain, and is only patchy in distribution over the cerebellum. In some animals examined twenty hours after inoculation the meningeal process is more diffuse; and in mice sacrificed twenty-two, twenty-six, and twenty-eight hours after infection the diffuse meningitis is most marked over the cerebellum, pons, and medulla. In one animal examined twenty hours after inoculation the dorsal part of the spinal cord is more involved than the ventral. In one mouse killed thirty-two hours after injection the character of the meningitis is different: it is focal in distribution, and the infiltrates consist more of round cells than of polymorphonuclear leukocytes.

In our opinion the accentuation of the meningeal process in the posterior fossa, a phenomenon seen also in human pathology, has to do with the drainage of cerebrospinal fluid from the ventricular system through the foramina of Luschka and Magendi into the posterior fossa. As will be further discussed,

Discussion

most of the experimental animals reveal a marked involvement of the ventricular system; and it is reasonable to believe that pus and infectious material would first be drained thence into the subarachnoidal spaces of the posterior fossa. These cisterns are normally large and this fact may be another reason for the marked accumulation of exudate there. The anatomical situation of the large cistern may also explain another of our findings. Reference is made to the more marked involvement of the dorsal aspect of the spinal cord as opposed to the ventral part, a finding also observed by Wertham (25) in his material.

The close relationship between the subarachnoidal spaces in the posterior fossa and the ventricular system is shown by the experiment of Stewart (23), who also noted frequent infection of the central canal in dogs infected by cisternal puncture.

Involvement of the ventricular system with ventriculitis and accumulation of pus in the ventricles is present in animals sacrificed as soon as twelve hours after inoculation. This fining, although often seen, is by no means constant: for instance, mice killed twenty or twenty-six hours after the beginning of the experiment fail to reveal changes in the ventricles. In some instances

in animals examined twelve or twenty-eight hours after inoculation there are a distension of the ependyma and periventricular necrosis with involvement of the surrounding subependymal tissue by polymorphonuclear leukocytic infiltrates. In some animals (twenty hours) no infiltrates are seen in the necrotic zone; and in general there is a discrepancy between the severity of necrosis and the mild leukocytic infiltration. The infiltrates are usually scattered but occasionally are also seen perivascularly in or around necrosis (twelve hours after inoculation). Similar observations have been reported on the involvement of subependymal tissue. Essick found an excoriation of the ependymal and the subependymal tissue when the ventricles were involved (9). No satisfactory explanation can be offered for the ventriculitis. One possibility is that the ventricular system was infected by the passing of the needle during inoculation. We do not have statistically significant data either to prove or to disprove this mode of infection. Another possibility has already been mentioned: extension of the meningeal process into the ventricular system. Still another explanation for the occurrence of ventriculitis would be to assume an extension of the inflammation from the subependymal tissue into the

ventricles. However, no significant involvement of the subependymal tissue is ever seen without ventriculitis and subependymal necrosis. In addition, in those instances where there are exudates in the subependymal tissue, they are slight compared to the ventriculitis and the extensive necrosis.

Involvement of the cerebral cortex has been mentioned in animals examined twelve hours after inoculation. In many areas scattered polymorphonuclear leukocytes can be observed in the cerebral cortex. In animals killed eighteen hours after the beginning of the experiment small foci of bacteria are seen with a weak leukocytic response. In animals sacrificed subsequently, bacteria are often seen, usually associated with infiltrates of polymorphonuclear leukocytes. To assume that most of these cortical infiltrates represent extensions of the meningitis would not be correct, because all animals were inoculated intracerebrally, and the brain tissue was thus opened to invasion by bacteria and toxic products. However, in some instances there is clear cut morphological evidence of meningeal extension into the underlying cortex: cortical and meningeal infiltrates are intimately connected; or from a heavy meningeal infiltrate a vessel surrounded by leukocytes penetrates the

underlying cerebral cortex. In some instances between closely related meningeal and cortical infiltrates an apparently intact external limiting membrane can be seen, while in other areas this has been partially destroyed.

Abscesses are seen in animals sacrificed as early as twelve hours afte inoculation. They are located in the base of the brain. In animals examined twenty hours after inoculation small fresh abscesses are seen scattered throughout the central nervous system. These abscesses are better circumscribed than those seen in the twelve hour animal. The longer the animal survived the more abscesses can be seen in the central nervous system.

Ayer found that abscess formation was rare in the cortex twelve hours after the onset of his experiment (3). Our findings are not in agreement with the observations of Essick (9), who reported that in experimental infection of the meninges the exudate is usually restricted to the perivascular spaces of the cortical vessels and that extension from the vessels is only seen in the presence of thrombosis. As already mentioned, in the experimental animals examined the inflammatory process is not only not restricted to the perivascular spaces but is remarkably expanded into the surrounding

underlying cerebral cortex. In some instances lesions
closely related ventricular and cortical hemorrhage
an apparently intact arachnoid limiting membrane can
be seen, while in other cases this has been completely
destroyed.

Abscesses may occur in orbital affections
early as twelve years after inoculation. They are
located in the base of the orbit. In thirty problems
twenty bombs after inoculation only four cases
are seen positive but the general process
system. There is decrease now before discovery but
than there were in the twelve hour animal. The
longer the earlier survived but some pictures can
be seen in the cerebral nervous system.

Ayer found the disease [illegible]
the cortex brain for three one month of [illegible]
experiment (?). Four fighters may not in consonance
with the observations of Smith (?), the reaction
that in experimental infection of the animals [illegible]
exudate is usually restricted to the [illegible]
areas of the cerebral cortex and [illegible]
from the vessels is only then [illegible]
thrombosis. In chronic conditions, as the cases
round defects [illegible] the [illegible]
not only for multiple [illegible]
but [illegible]

tissue. This may have to do with the particular arrangement of our experiment, especially with the intracerebral inoculation of the animals, and probably with the organism injected.

Two other prominent morphological features in this experiment are circumscribed hemorrhages and foci of necrosis, both of which are present in varying degrees of extension.

The hemorrhages are often symmetrical, as in an animal sacrificed twelve hours after the beginning of the experiment, in which hemorrhages are seen in the rhinencephalon. These hemorrhages are surrounded by a zone of necrosis and some scattered polymorphonuclear leukocytes in the parenchyma. In the same animal hemorrhagic foci are also seen in the meninges and in the granular layer of the cerebellum, where they are surrounded by polymorphonuclear leukocytes. Bilateral, symmetrical hemorrhages are seen in the cerebrum of another animal, sacrificed twenty hours after inoculation. Small hemorrhages in the cerebellar white matter are seen in a mouse killed at the twenty two hour stage. As already mentioned, these hemorrhagic foci are often surrounded by polymorphonuclear leukocytes. However, they do occur at times without any leukocytic reaction.

Scattered hemorrhages in the central nervous system have been described in experimental meningitis

by Ayer (3). Punctate hemorrhages and ecchymoses were seen by Essick (9) in experimental traumatic abscess. Further, Wertham (25) reported extravasations of blood into the parenchyma in infections. Several explanations have been offered in the literature for the genesis of hemorrhages in viral and bacterial infections. They have been thought to be artifacts due to removal of the brain at autopsy or to represent a terminal event of the infection in autopsy material. Another explanation, which we favor, has to do with the severity of the infection. Bodian (6) noted their occurrence in severe cases of poliomyelitis. Probably this explanation is also true for the present experiment, in which we deal with a very fulminant process. We do not know, and no explanation was found in the bibliography to explain why, some hemorrhages occur symmetrically in the brains of the experimental animals.

Necrotic foci of different sizes ranging from very discreet to very extensive are common in the examined animals. They are seen as early as twelve hours after inoculation. Often the necrosis is associated with bleeding, as in the thalamus and in the pons of an experimental animal killed at the twelve hour stage. Quite often they are symmetrical, as seen, again in an animal sacrificed at twelve

hours after the beginning of the experiment, in the region of the fourth ventricle. Near the margin of the necrotic zones polymorphonuclear leukocytes can very frequently be detected. In one animal sacrificed twenty eight hours after inoculation a necrotic focus is seen in the granular layer of the cerebellum, a finding not seen in the other experimental animals. Necrosis is very often associated with ventriculitis. The following changes are observed around the affected ventricular system, from the center to the periphery: 1) a pale zone of necrosis in the immediate subependymal tissue; 2) a hemorrhagic zone with polymorphonuclear leukocytes; 3) a zone of swollen astrocytes and, finally, in the periphery 4) a zone of microglial proliferation. Some microglia cells are also seen in the third zone. The extension of the necrotic zone around the ventricular system reveals variations in size from animal to animal. A similar zonal arrangement can be ovserved around the inoculation track. Here, again, the necrotic zone reveals variations from case to case. A special susceptibility of rhinencephalic centers to necrosis cannot be observed in the experimental animals. Hurst (13) saw in experiments with viruses in lower mammals a necrosis of the rhinencephalon which was absent in higher mammals. He therefore concluded

that the necrosis is, "characteristic of a certain type of animal host, rather than of the action of a particular virus" (13). We cannot find a clear predisposition of any area to necrosis in the experimental animals. Generally speaking, in any part of the central nervous system necrotic foci can be seen with irregular variations in number and extent from case to case.

With the exception of one animal sacrificed thirty-two hours after inoculation all mice examined reveal infiltrates composed of polymorphonuclear leukocytes. Some of these inflammatory lesions occurring in the cortex have already been discussed. Similar infiltrates are seen constantly around the inoculation tract and deep in the brain parenchyma. A region of the central nervous system in which infiltrates are seen in almost all animals is the cerebellum, especially the molecular layer. The majority of these infiltrates are seen in a scattered fashion, with or without clear relation to vessels, or in the form of more or less dense aggragates. Perivascular infiltrates composed of polymorphonuclear leukocytes are seen in animals sacrificed twelve hours after inoculation. However, many experimental animals shown no perivascular infiltrates. Most perivascular infiltrates are related to the abscesses,

to the diffuse infiltrates seen in the meninges and deep in the parenchyma, or to the region of inoculation, or to the ventriculitis. In one animal sacrificed twenty hours after the beginning of the experiment infiltrates are seen bilaterally and symmetrically and seem to follow fiber tracts. That pre-existing structures of the brain, such as myelin fibers, may have a formative influence upon such phenomena as demyelinization or the spread of hemorrhage, has recently been emphasized (7,18); while Essick (9) found a tendency of the infection in experimental traumatic abscess to follow fiber tracts.

While no evidence for a topical predisposition to necrosis is found in these animals, our findings coincide with those of Ayer (3), who reported that polymorphonuclear leukocytic infiltrates were a constant finding in the molecular layer of the cerebellum. Similarly, there seems to be a tendency for the dorsal half of the cord to be markedly more affected than the ventral half, a finding that corresponds with the report of Stewart (23).

The relation of polymorphonuclear leukocytes to hemorrhage and necrosis has been discussed above.

The necrotic foci and the hemorrhages are probably due in large part to the virulence of the inoculant. A contributory factor may be the severe

edema and resultant increase of intracranial pressure. It is known that severe edema may cause necrosis of the sensitive myelin structures (15). It is also known that increased intracranial pressure may produce hemorrhages, and foci of necrosis the brain tissue is pale and loculated. Within such foci, which are interpreted as edema, the cellular elements reveal regressive changes. The number of nerve cells is markedly reduced; and some still-existing neurons are in the process of lysis. The oligodendroglia cells are markedly reduced in number; and some which are still present reveal all stages of lysis and rhexis. In such necrotic zones the astrocytes reveal varying degrees of swelling and marked pallor of their nuclei. In the astrocytic nuclei the chromatin material is greatly reduced and often only a few chromatin particles can be seen in the periphery of the nuclei, next to the nuclear membrane.

Similarly changed astrocytes are seen in animals sacrificed as soon as twelve hours after inoculation in relation to meningitis, often in the presence of an intact limiting membrane. In addition, these pale, swollen astrocytes are seen in the periphery of hemorrhagic and necrotic foci and in association with cortical leukocytic infiltrates. In some areas, for instance, adjacent to severe ventriculitis the

astrocytes are very pale, their nuclei containing almost no chromatin particles, and are obviously in a state of disintegration. Similar changes are seen in many astrocytes in the molecular layer of the cerebellum. These astrocytic changes are seen in all experimental animals examined. Astrocytic pallor is more consistent than nuclear swelling. With the exception of an animal sacrificed twenty-six hours after inoculation, no increase in the number of astrocytes can be observed with certainty. In this animal the astrocytes are increased in number beneath areas of cerebellar meningitis.

In some areas beneath meningitis the astrocytes show changes ranging from pallor with relatively well preserved processes to minimal or absent swelling with decreased, absent, or short, clumped processes. Astrocytes show thick, fragmented, few or no processes next to abscesses and infiltrates. In alternate hematoxylin-eosin sections the same cells reveal pallor and swelling. Similar changes of astrocytes are seen in some ependymal regions revealing necrosis. In general, where there are polymorphonuclear leukocytes the astrocytes reveal very marked degrees of change in their processes. The staining of processes improves with distance from the foci described previously. In the vicinity of abscesses many

astrocytes are very rare, their nuclei (sometimes
almost no chromatin material. Of the processes of
a state of disintegration. Slightly changed are
in many astrocytes in the molecular layer of the
cerebellum. These astrocytic changes are seen in
all experimental epileptic animals. Likewise slight
is more significant than nuclear modification.
exception of an altered reactivity (staining-
after inoculation, be increase in the number of astro-
cytes can be observed with microscope. In this manner
the astrocytes are important in maintaining
of cerebellar neurons.

In some cases hematoxylin-eosin
show changes resembling those called as pyknotic
well preserved (because they stained as strongly
with decreased, dense, or shunt. Changed
Astrocytes show large
next to blood vessels and nerve cells.
Hematoxylin-eosin
major and modified.
are seen in some stained
In general, there
cytes the
changes in their
Therefore
creased in

astrocytes reveal fragmentation of processes and a moth-eaten appearance of the cell body, or, if the cells are relatively well preserved, swelling of the cell body with irregular processes.

With the silver techniques one can obtain considerably more information about astrocyte changes than with hematoxylin-eosin stain. All astrocytic changes, whether they reveal fewer or irregular processes, fragmentation or absence of processes, appear alike in hematoxylin-eosin; i.e., they reveal swelling and pallor.

Swelling and pallor of astrocytes are well known changes taken by many pathologists as criteria of edema. Very often these morphological changes are the only manifestations of edema since edema fluid, especially when low in protein, is not demonstrable with our usual histological techniques. Scholz (21) interpreted the presence of swollen astrocytes beneath meningeal infiltrates as indicative of edema. This interpretation has seemed reasonable because pale, swollen astrocytes are seen in such conditions as the neighborhood of tumors (11, 12, 14, 19, 20), around hemorrhage (1, 2, 15, 22), in trauma (24, 26, 27, 28), in arteriosclerosis, in infections, etc., in all of which edema is a prominent morphological feature.

The question arises, of course, as to whether this opinion is correct. It may very well be that the cellular swelling represents an alteration due to toxic or abnormal metabolic products and has little or nothing to do with edema fluid.

Collias and Manuelidis (7) described astrocytic changes consisting of pallor and swelling around puncture wounds of the brain; yet the pale, swollen astrocytes described by these authors appeared entirely different in silver preparations from the astrocytes described in our animals. In their work, astrocytes maintained more processes, which were thicker, better preserved, and more fully stained with silver carbonate. In the mice inoculated with hemolytic streptococci in this experiment, processes are fragmented; yet in hematoxylin-eosin preparations the cell bodies resemble those of the previous experiment. The question arises as to whether we are dealing with a process different from edema or whether some pathogenic factor in addition to edema is acting on astrocytes in such a way as to cause fragmentation and disintegration of the cells.

Experience from human pathology indicates that inflammatory processes of the central nervous system, regardless of the nature of the infectious agent, cause an increase in brain volume. Such brains weigh

more and feel softer than normal brains. Returning to our experiment, there is abundant evidence that components with a larger particle size than protein, such as red cells and lymphocytes, can escape from the blood stream and infiltrate the brain tissue. It is therefore reasonable to assume that there is a permeability disturbance for fluid and molecules such as protein, of considerably less size than blood cells.

In other words, the morphological changes observed in astrocytes do not at all exclude the presence of edema. Furthermore, in our experimental material, in addition to morphological evidence of edema, there are other signs indicating permeability disturbances of the vessels, hence suggesting a likelihood of edema. These are the loculation and pallor seen, for instance, around hemorrhages and in the vicinity of ventriculitis.

However, the fact that in such conditions as the traumatic bleeding, arteriosclerosis, and vicinity of tumors previously mentioned there are astrocytes with well preserved processes, while in the present experiment there is a strong tendency toward process fragmentation and destruction, suggests that factors other than edema are operating. The most obvious possible other factor is a toxic one, which seems to cause first a reaction of astrocytes and then their

disintegration. The fact that astrocytes in the immediate neighborhood of infiltrates, abscesses, etc., reveal marked changes as compared with those more removed from the lesion without regard to the regional vasculature also speaks in favor of our interpretation.

In the literature there is a paucity of information concerning astrocytic changes in experimental infections. In the viral encephalitides pallor and swelling of astrocytes have been seen and have been interpreted as manifestations of accompanying edema (18). A similar interpretation has been given to changed astrocytes in bacterial diseases (22). However, in these papers the astrocytes were described only with hematoxylin-eosin, Nissl, and hematoxylin-van Giesson stains, and not with the silver technique of Hortega.

The most acute change of astrocytes is fragmentation of processes with little or no swelling (8). Regressive changes have been described in astrocytes as clasmatodendrosis of processes with pyknosis and lysis of nuclei, followed by loss of processes, rounding of the cell body, and accumulation of fat in the cytoplasm.

All these changes are seen in our experimental animals, with the exception of fat, because no specific stain was undertaken.

In experimental brain wounds changes ranging from swelling to complete necrosis with fragmentation of processes, etc., have been described (4, 9). The closer the astrocytes were to the zone of bleeding and infiltrate next to the wound, the more marked were the regressive changes (ibid). Qualitatively similar observations hold for animals of the present experiment. The astrocytic hypertrophy seen in experimental wounds cannot be detected in our animals inoculated with hemolytic streptococci. Neither are amitotic and mitotic division generally seen in the experimental animals. There are some rare exceptions in which, near hemorrhages, amitotic divisions of astrocytes are seen; and in one instance astrocytes of the cerebellar molecular layer appear increased in number, though without signs of mitotic or amitotic division.

In animals sacrificed twelve hours after inoculation some twisted microglia are seen below areas of meningitis and throughout the forebrain, while some rectangular forms are seen in the molecular layer of the cerebellum. Furthermore, a mild microglial proliferation is seen in all those zones in which astrocytes are changed, extending beyond the zone of astrocytic reaction. Many reactive microglia are seen in the vicinity of the inoculation tract. In

In experimental brain wounds changes ranging from swelling to complete necrosis with fragmentation of processes, etc., have been described ([illegible]). The closer the astrocytes were to the [illegible] and infiltrate next to the wound, the more marked were the regressive changes (1918). Qualitatively similar observations hold for animals of the present experiment. The selective hypertrophy seen in experimental wounds cannot be detected in our animals inoculated with hemolytic streptococci. Neither are amitotic and mitotic division commonly seen in the experimental animals. There are some rare exceptions in which, near the wound, mitotic divisions of astrocytes were found and in the [illegible] astrocytes of the cortex [illegible] increased in number, though evidence of mitotic or amitotic division.

In animals sacrificed twelve hours after inoculation some isolated microglia are seen below the meninges and throughout the cortex. Rod cells in rectangular forms are seen in the molecular layer of the cerebellum. Fourth month, a mild diffuse proliferation is seen in all layers [illegible] astrocytes are enlarged, [illegible] astrocytic reaction. [illegible] seen in the vicinity of the [illegible].

only one instance is a module of proliferated microglia seen, this in the cerebral cortex of an animal dying twenty hours after inoculation. In general, there is a strong proliferation of microglia surrounding abscesses; but the most consistent and heaviest proliferation is seen in the cerebellar molecular layer.

The microglial changes occurring in a variety of viral encephalitides have recently been reviewed (16). The microglia cells in viral diseases reveal a great increase in number, as opposed to the present experiment, in which the microglial cells show a relatively limited proliferation and alteration more in the form of changes in size and shape. However, it should be emphasized that in the present experiment reactive microglia appear as soon as in an experiment undertaken with poliomyelitis and West Nile virus infections, i.e, twelve hours after inoculation (17). In noninflammatory experimental conditions microglial proliferation has been described in brain wounds twenty to twenty-four hours after injury (4,7). In a case of purulent meningitis diffuse proliferation of microglia has been described in the molecular layer of the cerebellum early in the course (25), while in an experiment involving the injection of staphylococci proliferation of microglia has been detected within twelve hours (5).

There are several possible explanations for the lesser degree of microglial proliferation seen in this experiment as compared with other material, especially the viral encephalitides. Among these is the fact that the present infection may be characterized as super acute, as animals usually died well before twenty-four hours; hence it is likely that there was insufficient time for the full expression of a proliferative tendency by the microglia. Another possibility relates more directly to the great virulence of the inoculant, which may have had a suppressive effect on the microglia.

SUMMARY

White mice were injected intracerebrally with cultures of alph-hemolytic streptococci. Most animals died within twenty-four hours.

The material presented is a histopathological study of nervous tissue removed after sacrifice of animals which appeared to be in the terminal stages of infection at timed intervals following injection.

Purulent meningo-encephalitis is a constant finding; and its character, distribution, and relation to other lesions are discussed and described. Less constant but frequent findings include ventriculitis and periventricular necrosis, cortical and perivascular infiltrates, necrotic foci, abscesses, and hemorrhages; and these are similarly described and discussed.

The significance of astrocytic swelling and pallor are discussed in the light of experimental findings, as is a relatively mild but constant proliferative tendency of microglia.

BIBLIOGRAPHY

1. Alajouanine, T.: Oedeme Cerebromeninge par hypertension arterielle, Rev. Neurol., 1947, 79:263-273
2. Anton, G.: Gehirnoedem und Compression, In Flatau, E., Jacobsohn, L., and Minor, L.: Handbuch der pathologischen Anatomie des Nervensystems.
3. Ayer, J.B.: A Study of Experimental Meningitis. II A Pathological Study of Experimental Meningitis from Subarachnoid Inoculation. Monograph of the Rockerfeller Inst. for Med. Research, 1920.
4. Baggenstross, A.H.; Kernohan, J.W.; and Drapiewski, J.F.: The Healing Process in Wounds of the Brain. Am. J. Clin. Path. 1943, 13:333-343.
5. Bazgan, J., and Enachescu, D.: Recherches Experimentales sur la Microglie. Ann. Anat. Pathol., Med-chir. 1929, 6:43-56.
6. Bodian, D.: Histopathologic basis of Clinical findings in poliomyelitis. Am. J. Med. 1949, 6:563-578.
7. Collias, J.C. and Manuelidis, E.E.: Histopathological Changes Produced by Implanted Electrodes in Cat Brains. Journal of Neurosurgery, 1957, 14:302-334.

1. [illegible]

2. [illegible]

3. [illegible]

4. [illegible]

5. [illegible]

6. [illegible]

7. [illegible]

8. Cone, W.: Acute Pathologic Changes in Neuroglia and in Microglia. Arch. Neurol. and Psychiat. 1928, 20:34-67

9. Essick, C. R.: Pathology of Experimental Traumatic Abscess of the Brain, Arch. Neurol. and Psychol. 1919, 1:673-704

10. Globus, J.H.: Glia Response in Chronic Vascular Disease of the Brain. Arch. Neurol. and Psychiat. 1928, 20: 14-33

11. Greenfield, J.G.: The Histology of Cerebral Oedema Associated with Intracranial Tumors (with special Reference to Changes in the Nerve Fibers of the Centrum Semiovale). Brain, 1939, 62:124-152.

12. Häussler, G.: Hirndruck, Hirnödem, Hirnschwellung. Zentralb. F., Neuchir., 1937, 2: 247-261, 328-336

13. Hurst, E.W.: The Histology of Equine Encephalomyelitis. J. Exp. Med. 1934, 59:529-542.

14. Jacob.H.: Über diffüse Markdestruktion im Gefolge eines Hirnodems, Ztschr. f.d. ges. Neur. u. Psychiat. 1940, 168:382-395.

15. Le Beau, J.: L'oedeme du Cerveau. Son Role dans L'evolution des Tumeurs et des Abscesses Intracraniens, (Thesis) Paris, Recht, 1938, pp. 1-223

9. Crue, W.: Acute Pathologic Changes in Synovia and in Chronic Arthritis. Arch. Surg. 42:1-47 1928.

9. Smelck, G. D.: Synovitis of Gonorrheal Traumatic Origin of Long Duration. Amer. Urol. and Cutan. Rev. 32:113-120

10. Globus, J.H.: Old Gonorrhea in Chronic Arthritis Disease of the Joints. Arch. Neurol. and Psychiat. 1928, 20: 1-9

11. Greenfield, J.G.: The Histology of Synovial Tissue Associated with Inflammation (with special Reference to Changes in the Villi) Pathology of the Central Nervous System. London, 1927. 63:139-142.

12. Hanaoka, K.: Gonococcus, Urethritis, Prostatitis, Epididymitis, Cystitis. Jour. Infect. Dis. 1: 1-. 1904.

13. Hurst, E.W.: The Pathology of Acute Gonorrhoeal Arthritis. J. Am. Med. Ass. 1920, 74: -.

14. Jacobi, H.: Three Cases of Gonorrheal Rheumatism Complicated by Endocarditis. Trans. Am. Gyn. Soc. 1884, 9: 1-.

15. Le Fevre, J.: Pathology of Synovitis. The Bull. Johns Hopkins Hosp. 1 (Jan-Jul) Part 1. 1890. pp. 1-.

16. Manuelidis, E.E.: General Histopathological Aspects of Some Experimental Viral Encephalitides. In. Henke Lubarsh. Hanbuch der Speziellen Pathologischen.

17. Manuelidis, E.E. and Chernow, M.A. Study of Microglial Reaction in Experimental Poliomyelitis and West Nile Encephalitis. In preparation.

18. Manuelidis, E.E. and Haymaker, W. The Influence of Edema and Plasmatic Fluid on Brain Tissue in Viral Encephalomyelitides and Allied Disorders (Epidemic Hemorrhagic Fever, Japanese Encephalitis, Russian Spring-Summer Encephalitis, and Postinfectious Encephalitis) Unpublished data

19. Scheinker, I.: Über das gleichzeitige Vorkommen von Hirnschwelling und Hirnödem bei einem Falle einer Hypernephrom metastase des kleinhirns. Deutsche Ztschr. f. Nervenh. 1938, 148:1-16

20. Scheinker, M.I.: Neurosurgical Pathology. Springfield, Ill., Thomas, 1948, 1-10.

21. Sholz, W.: Histologische und topische Veranderungen und Vulnerabilitatsver haltnisse im menschlichen Gehirn bei Sauerstoffmangel, ödem, und plasmatischen Infiltrationen. Arch. f. Psychiat., 1941, 181:621-665.

16. Mannsdörfer, K.-H.: [illegible]

17. Mannsdörfer, K.-H.: [illegible]

18. Mannsdörfer, K.-H.: [illegible]

19. Mannsdörfer, K.-H.: [illegible]

20. Schneider, M.: [illegible]

21. Schulz, F.: [illegible]

22. Sholz, W.: Histopathologie von Storungen der Blut, Hirn, Schranke, insbesondere bei der Entzündung. Proc. II. Int. Cong. Neuropath. London 1955. Excerpta Medica. Sec. VIII, 1955, 8:842-844

23. Stewart, F.W.: Local Specific Therapy of Experimental Pneumoccal Meningitis, III Incidental Myelitis, Abscess, and Organisation of Exudates. J. Exp. M., 1928, 47:1-7.

24. Vincent. C., LeBeau, J., Guiot, G.: L'oedeme Cerebral en Neurochirurgie. Rev. Neurol. 1947, 79:273-276.

25. Wertham. F.: The Cerebral Lesions in Purulent Meningitis. Arch. Neurol. Psychiat. 1931, 26:549-582.

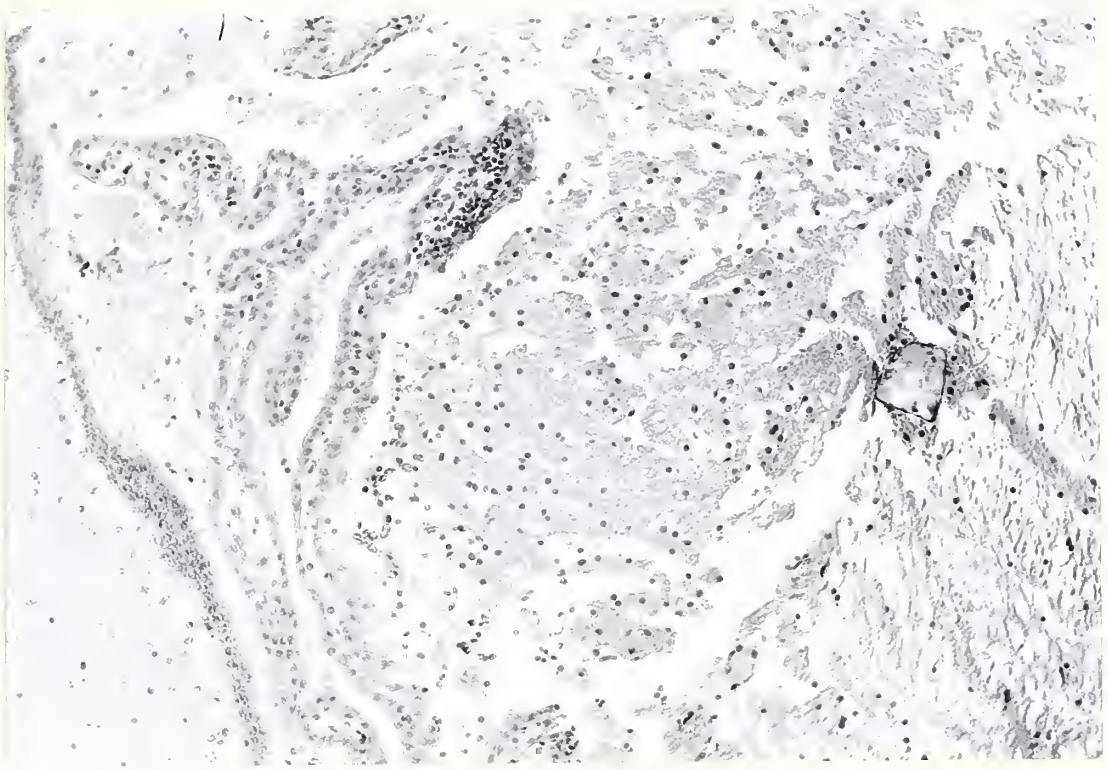
26. Zulch, K.J. Morphologische Veränderungen bei Hirnschwelling. Zentrabl, f. Neurochir., 1940, 5:166-174

27. Zulch, K.J.: Hirnodem und Hirnschwellung. Virchows Arch. 1943, 310:1-58.

28. Zulch, K.J.: Hirnschwellung und Hirnodem. Deutsche Ztschr. f. Nervenh., 1953, 170:179-208.

Fig. 1. Periventricular necrosis. H&E. X100.

Fig. 2. Swollen astrocytic cell bodies near periventricular necrosis. H & E. X440.



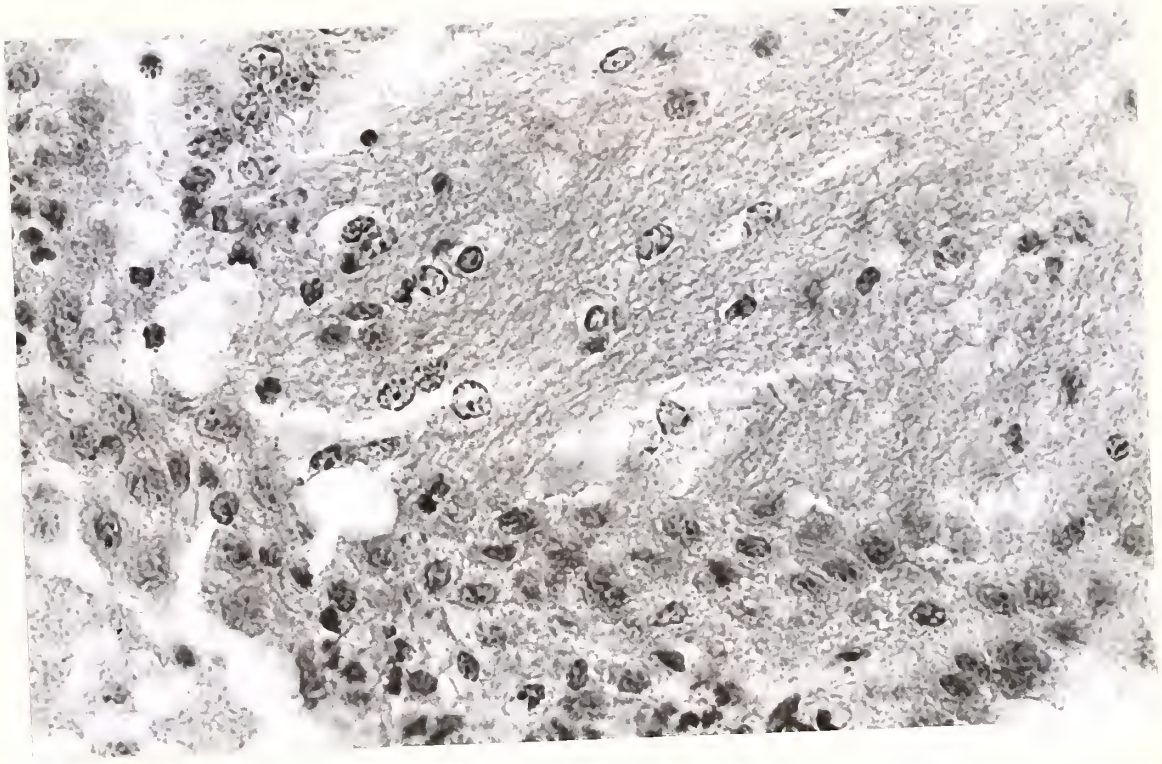
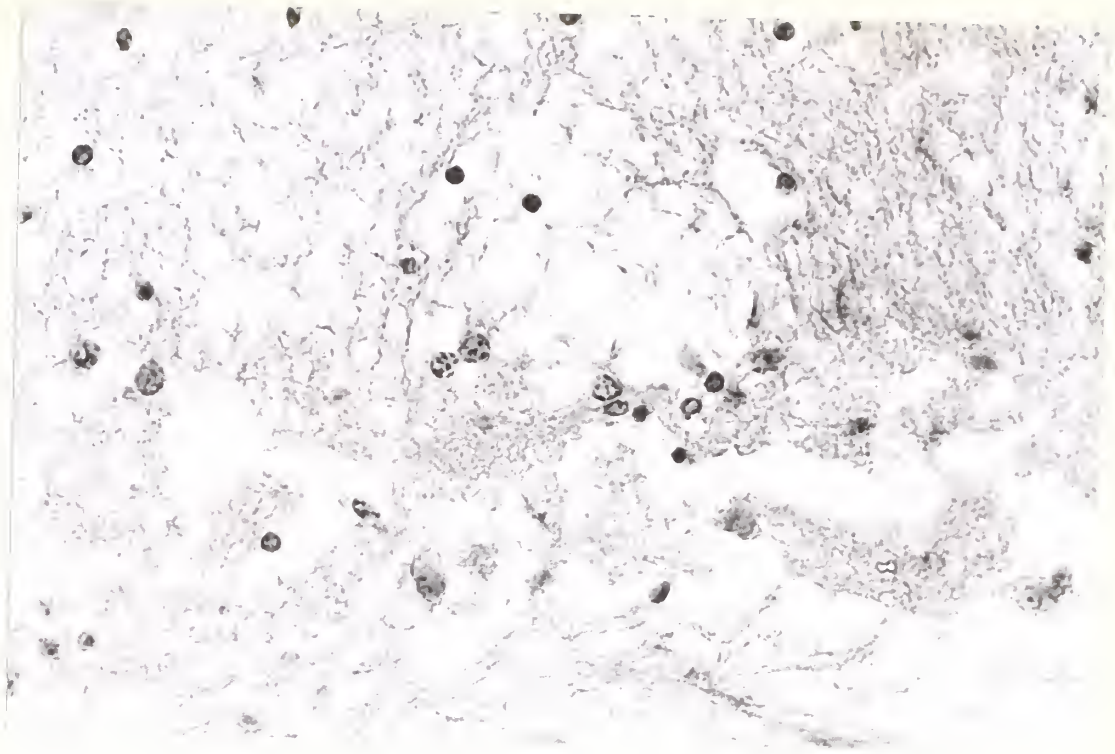


Fig. 3. Loculation in zone of periventricular necrosis. H & E. X400.

Fig. 4. Poorly preserved astrocytes beneath intense ventriculitis. Astrocyte stain. X440.



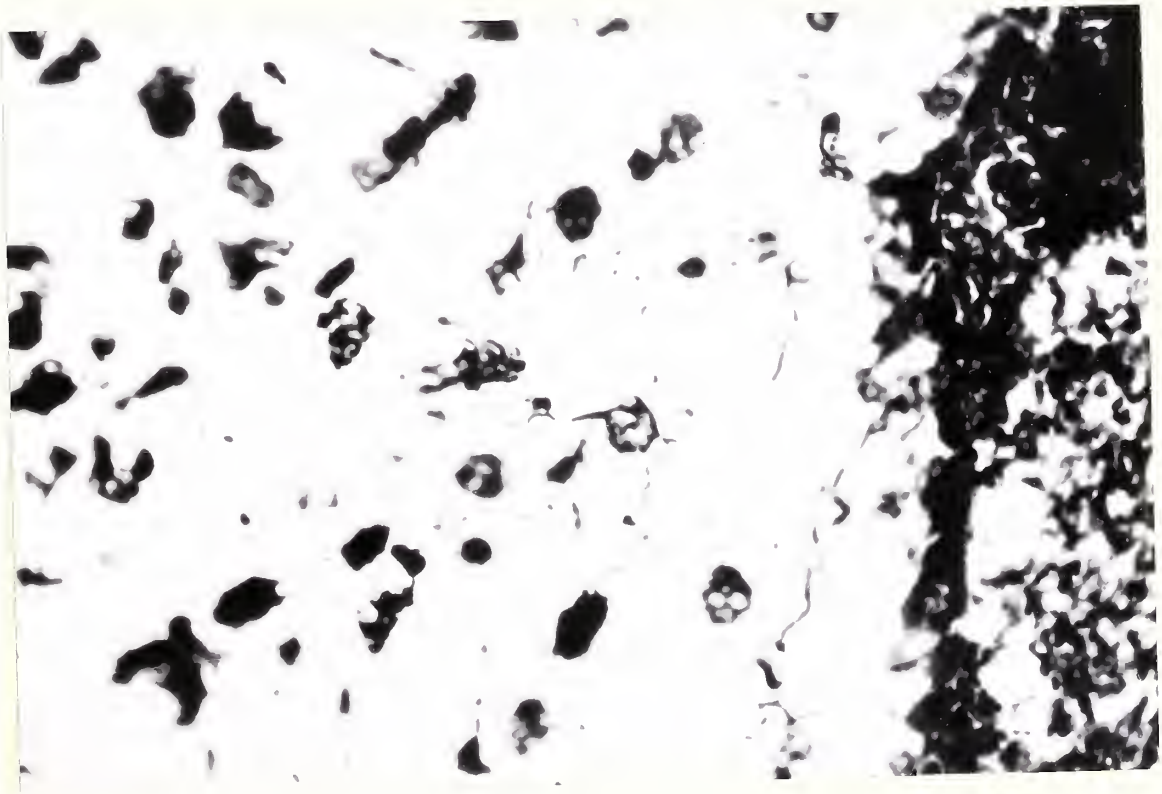
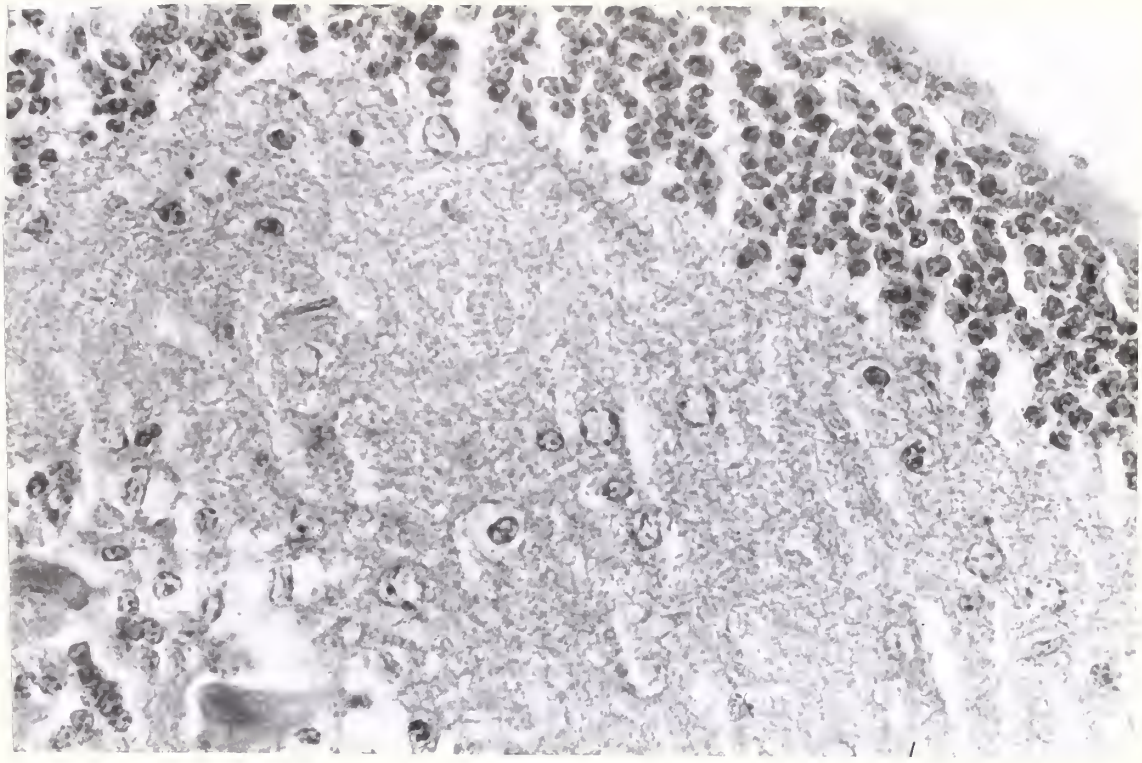


Fig. 5. Astrocytic swelling and pallor beneath intense meningitis. H & E. X400.

Fig. 6. Loss of astrocytic processes near inflammation in spinal cord. Astrocyte stain. X400.



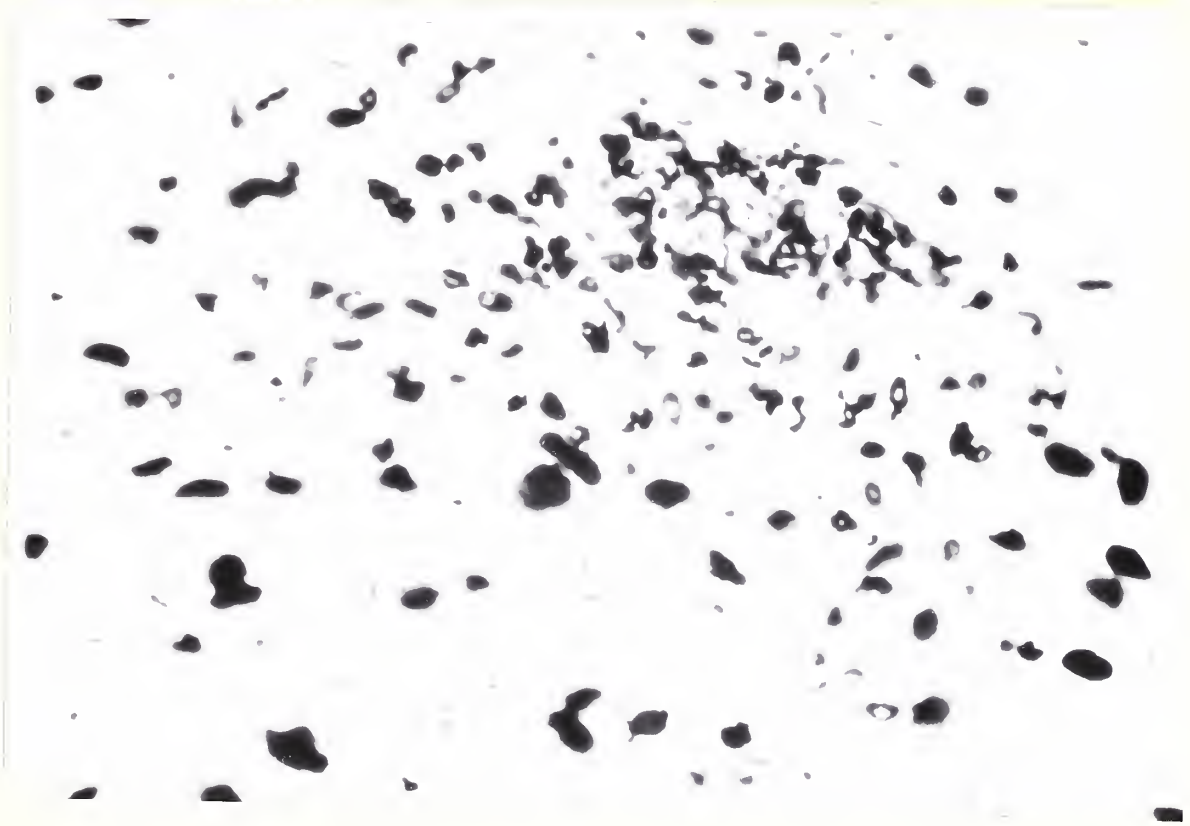
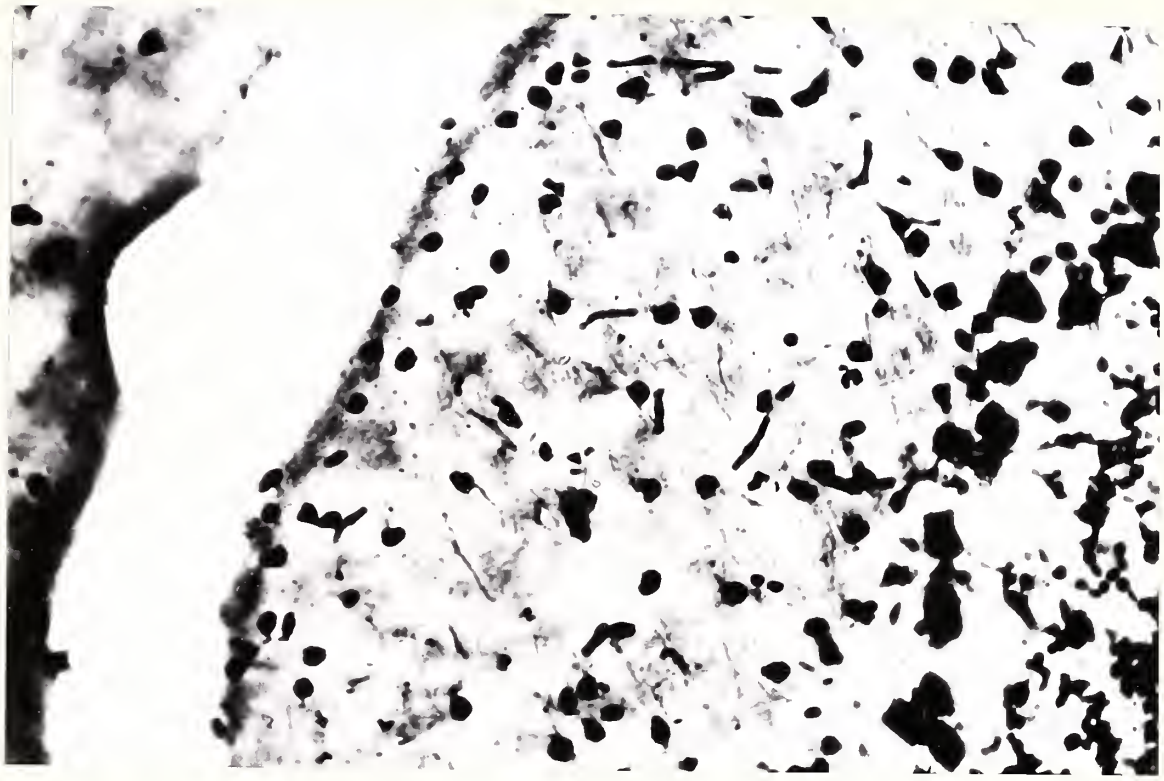


Fig. 7. Isolated microglial proliferation in molecular layer of cerebellum. Microglia stain. X300.

Fig. 8. Superficial abscess. Astrocyte stain. Low power.



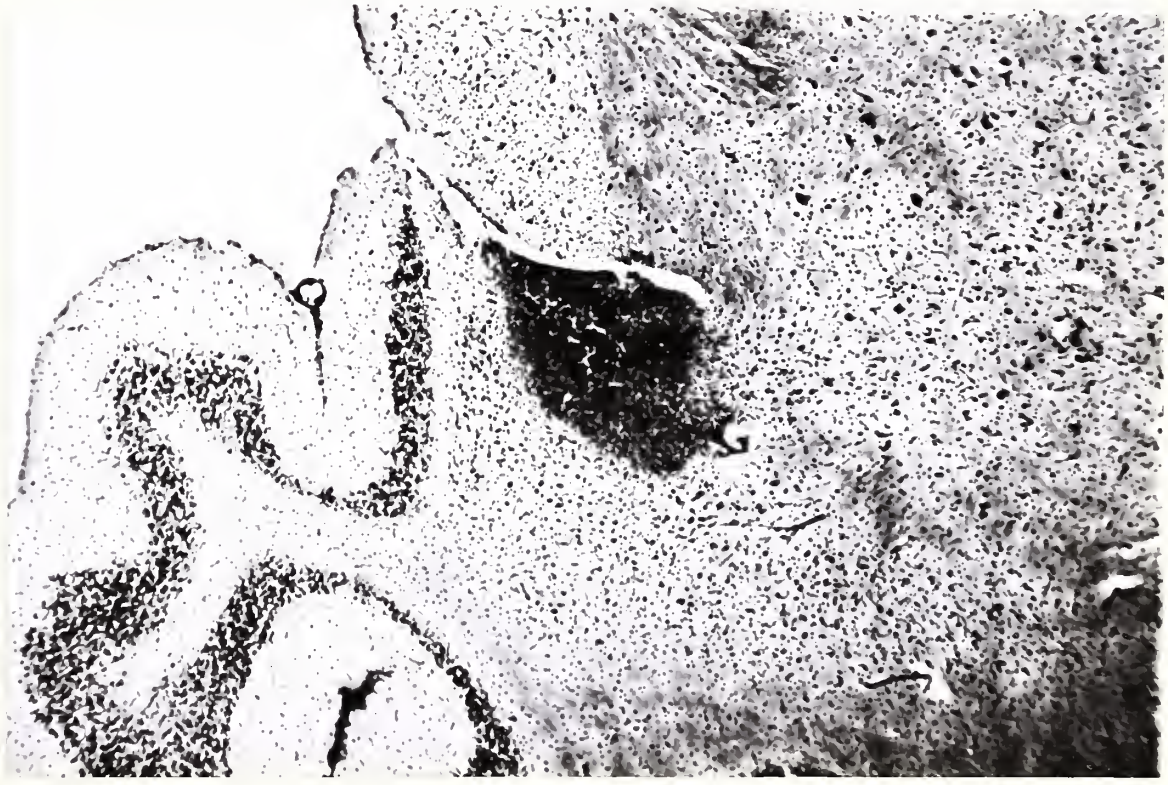


Fig. 9. Loss of astrocytic processes adjacent to intense ventriculitis. Astrocyte stain. X440.

Fig. 10. Relatively well preserved astrocytes beneath intact ependyma. Astrocyte stain. X440.

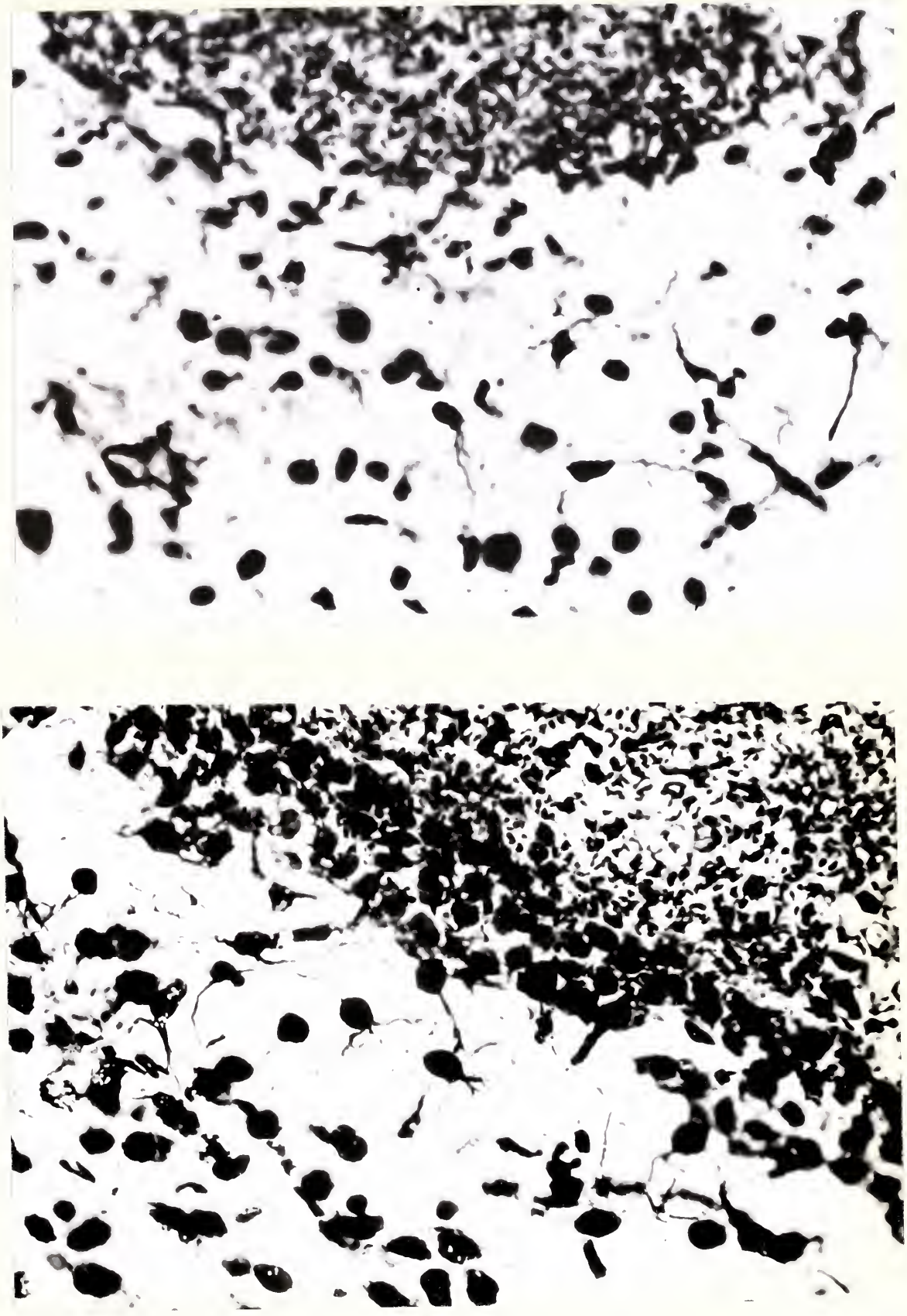
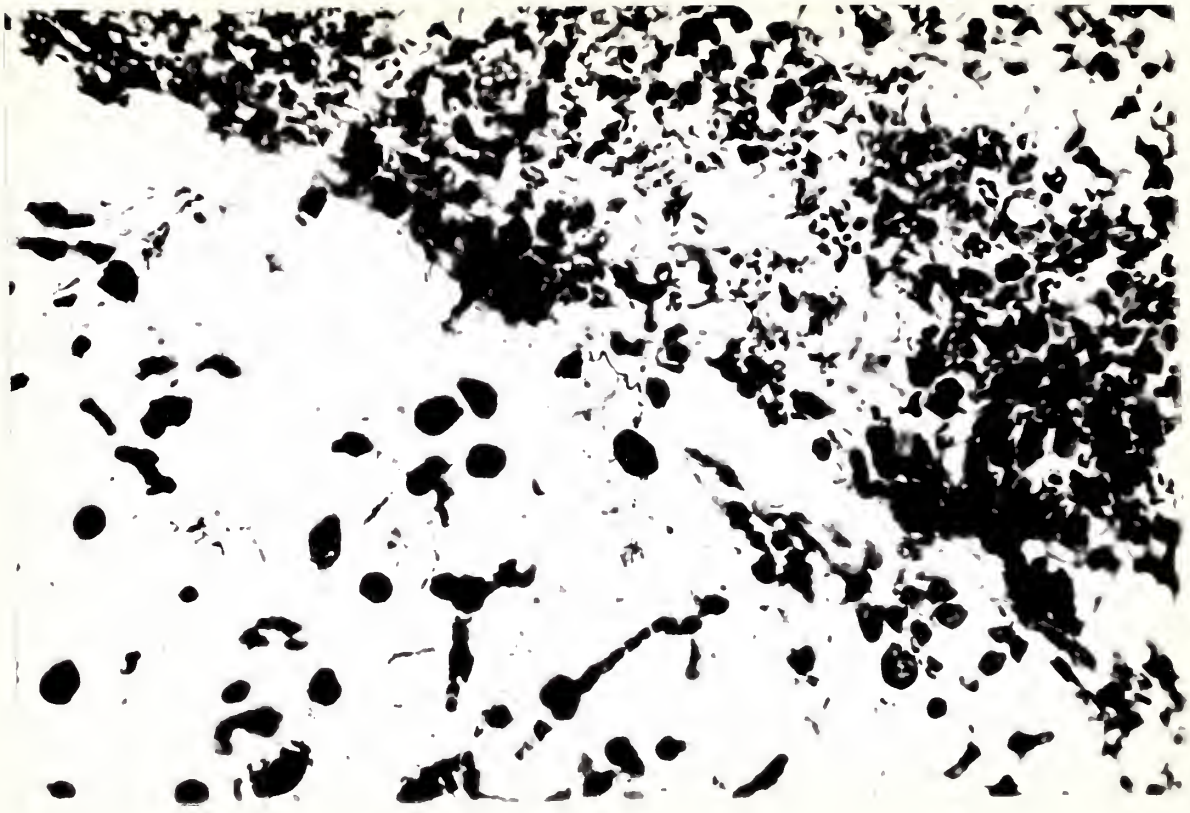


Fig. 11. Damaged astrocytes beneath damaged ependyma. Same section as Fig. 10. Astrocyte stain.

Fig. 12. Most marked degree of astrocytic changes beneath meningitis. Astrocyte stain. X440.



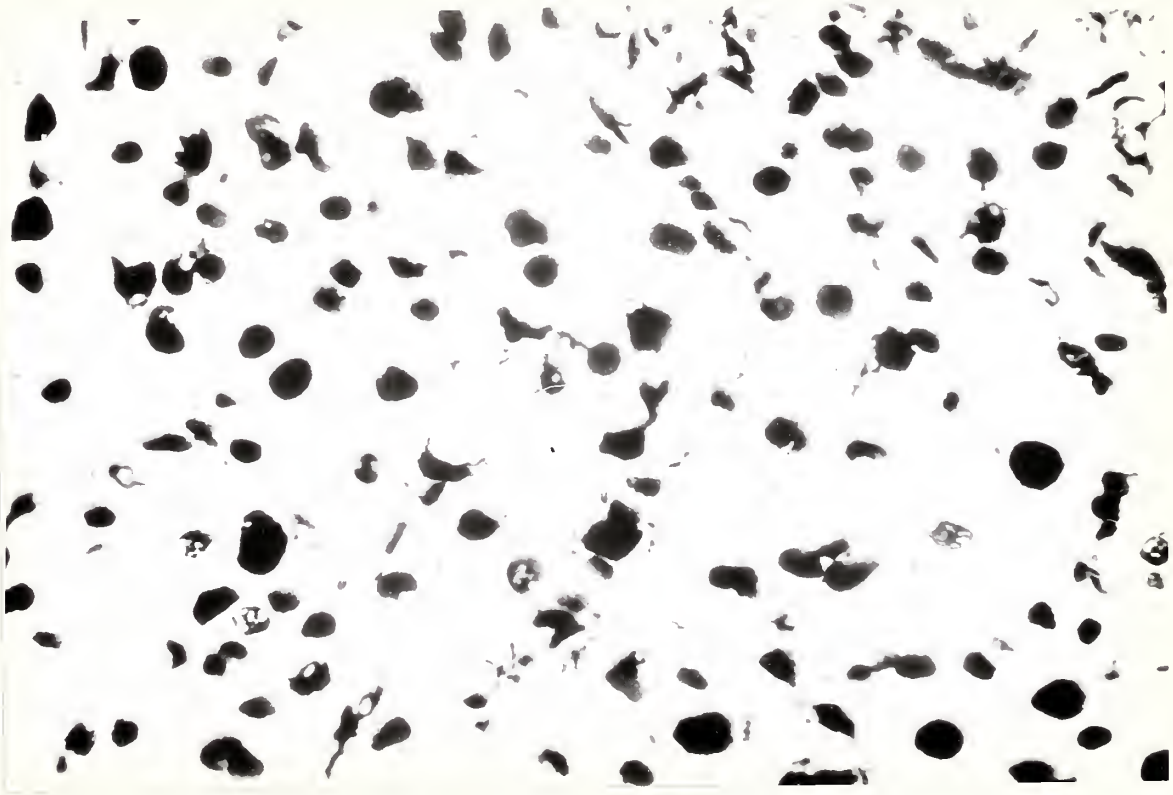
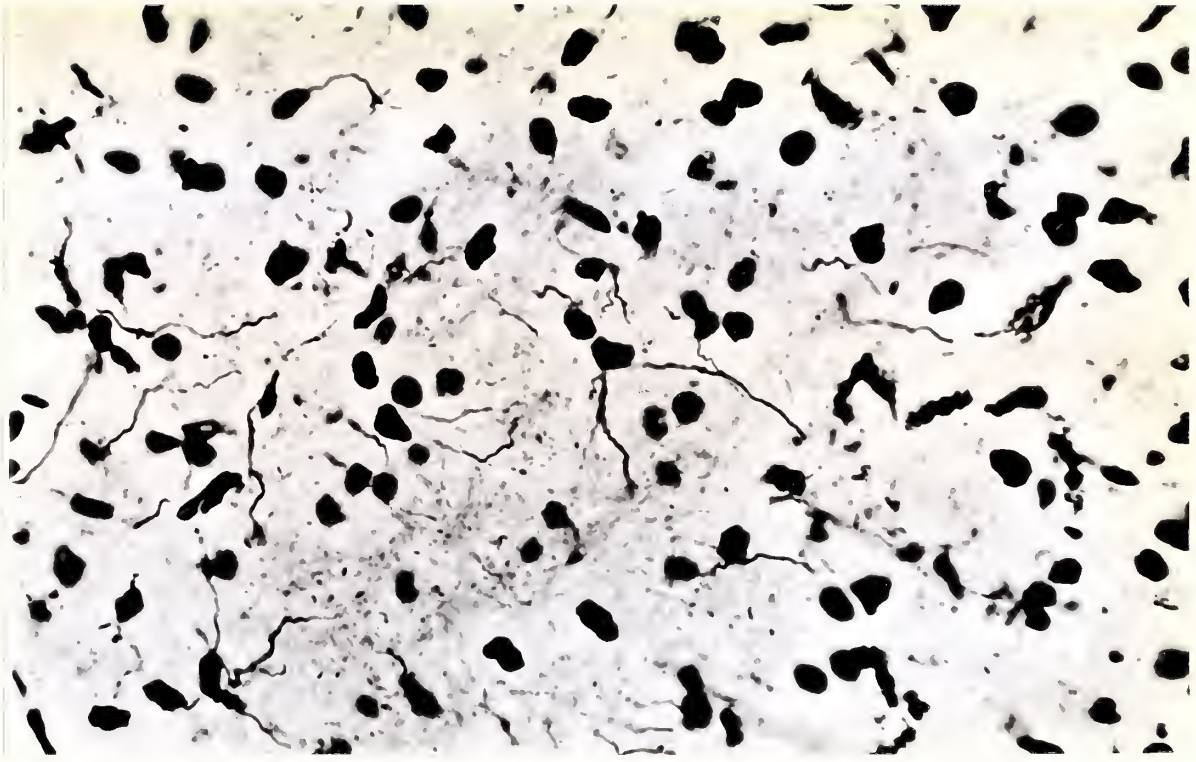


Fig. 13. Fairly well preserved astrocytes at margin of necrotic zone surrounding abscess. Astrocyte stain. X400.

Fig. 14. Astrocytic process fragmentation and cell body vacuolization immediately subjacent to meningeal infiltrate. Astrocyte stain. X440.



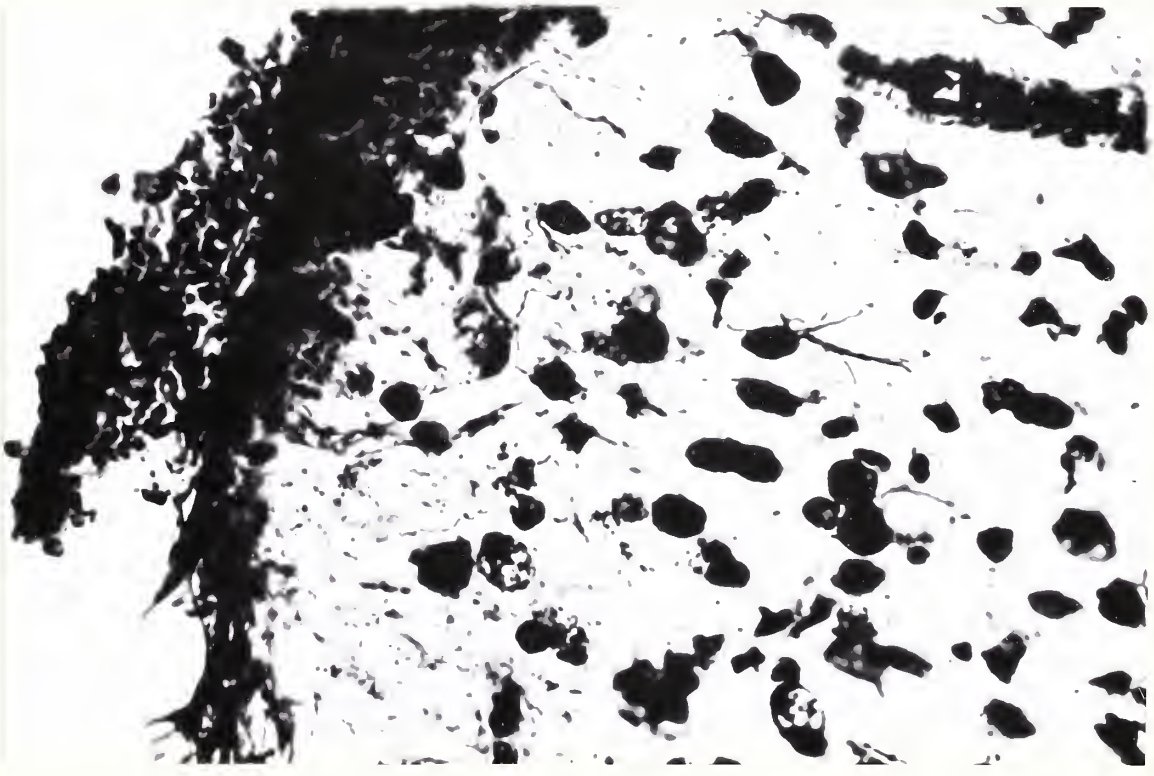
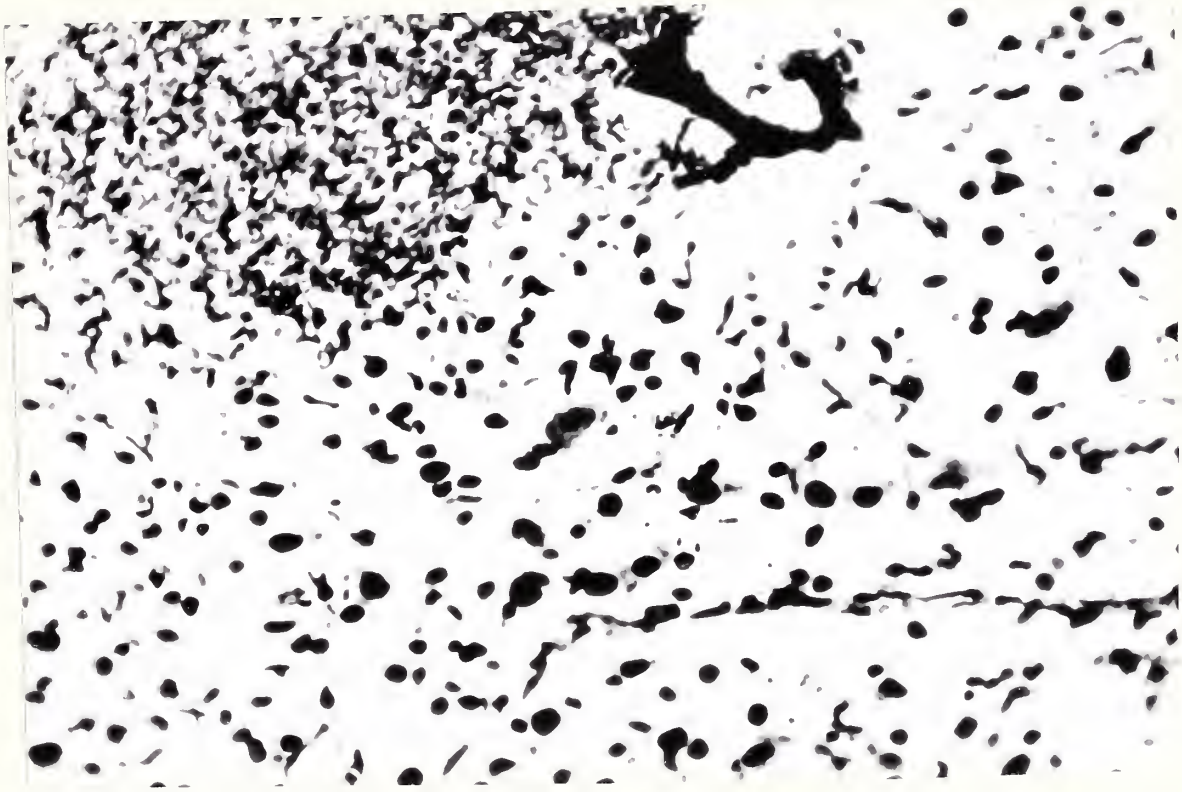


Fig. 12. Fibroblastic proliferation near abscess.

Aspergere stain. X100.

Fig. 15. Microglial proliferation near abscess.

Astrocyte stain. X200.



YALE MEDICAL LIBRARY

Manuscript Theses

Unpublished theses submitted for the Master's and Doctor's degrees and deposited in the Yale Medical Library are to be used only with due regard to the rights of the authors. Bibliographical references may be noted, but passages must not be copied without permission of the authors, and without proper credit being given in subsequent written or published work.

This thesis by has been used by the following persons, whose signatures attest their acceptance of the above restrictions.

_____

_____

NAME AND ADDRESS DATE